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Inventor(s):	Cruce et al
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Commissioner of Patents
Washington, DC 20231

Sir:

Preliminary Amendment Under 37 CFR 1.173(b)

In response to the Patent Issuance of 09 November 1999 (09.11.1999), please amend this US Patent as follows:

In The Drawings

Applicant is proposing to amend FIG. 8 under 37 CFR 1.173(b)(3). This amendment is to correct a misspelled word. Following is the proposed amendment in red.

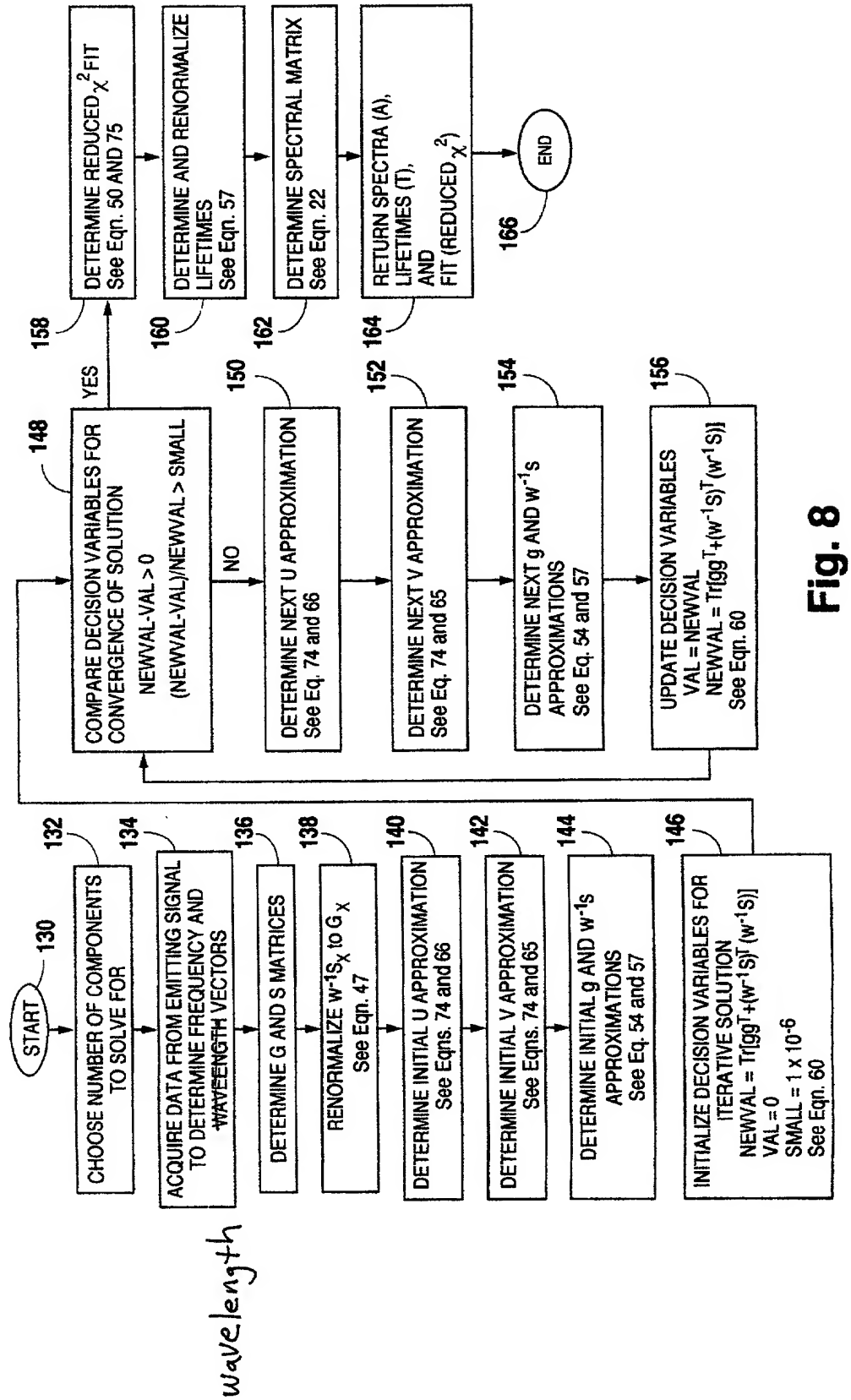
START
130
CHOOSE NUMBER OF COMPONENTS TO SOLVE FOR
132
ACQUIRE DATA FROM EMITTING SIGNAL TO DETERMINE FREQUENCY AND WAVELENGTH VECTORS
134
DETERMINE G AND S MATRICES
136
RENORMALIZE $w^{-1}S_\chi$ to G_χ See Eqn. 47
138
DETERMINE INITIAL U APPROXIMATION See Eqns. 74 and 66
140
DETERMINE INITIAL V APPROXIMATION See Eqns. 74 and 65
142
DETERMINE INITIAL g AND w^{-1}s APPROXIMATIONS See Eq. 54 and 57
144
INITIALIZE DECISION VARIABLES FOR ITERATIVE SOLUTION
NEWVAL = $Tr[gg^T+(w^{-1}S)^T(w^{-1}S)]$
VAL = 0
SMALL = 1 x 10^{-6}
See Eqn. 60
146
UPDATE DECISION VARIABLES
VAL = NEWVAL
NEWVAL = $Tr[gg^T+(w^{-1}S)^T(w^{-1}S)]$
See Eqn. 60
156
DETERMINE NEXT g AND w^{-1}s APPROXIMATIONS See Eq. 54 and 57
154
DETERMINE NEXT V APPROXIMATION See Eq. 74 and 65
152
DETERMINE NEXT U APPROXIMATION See Eq. 74 and 66
150
COMPARE DECISION VARIABLES FOR CONVERGENCE OF SOLUTION
148
NEWVAL-VAL > 0
(NEWVAL-VAL)/NEWVAL > SMALL
YES
NO
DETERMINE REDUCED χ^2 FIT See Eqn. 50 AND 75
158
DETERMINE AND RENORMALIZE LIFETIMES See Eqn. 57
160
DETERMINE SPECTRAL MATRIX See Eqn. 22
162
RETURN SPECTRA (A), LIFETIMES (T), AND FIT (REDUCED χ^2)
164
END
166
wavelength

Fig. 8

In The Specification

Applicant is amending the specification in this patent under 37 CFR 1.173(b) and 37 CFR 1.173(d). These changes include ministerial changes, grammatical changes, and mistakes made by the US PTO during the printing of the patent. Applicant is not adding new matter with these amendments.

Amend the specification with the selected replacement paragraphs on the following pages.

Paragraph at c. 2, l. 37-54.

In practice, we typically probe many different types of molecules at once with the excitation light pulse. FIG.2 illustrates the case where a single short excitation pulse of light 30 is absorbed by a sample of identical molecules all at once. The fluorescence decay curve 32 resulting from a typical fluorescence response of a sample of identical molecules is exponential in nature because not all of the identical molecules emits its fluorescence photon at precisely the same time. The exponential decay follows a mathematical function so that we can calculate the fluorescence lifetime, τ:

$$[I(t) = I_0 \bullet e^{-(t-5_0)/\tau}]$$

$$I(t) = I_0 \bullet e^{-(t-t_0)/\tau}$$

where t_0 is the time of the excitation pulse, I_0 is the initial fluorescence and I(t) is the observed fluorescence intensity as a function of time.

Paragraph at c. 5, l. 30-43.

FIG.5 is a block diagram of the generator component 54 of the present invention for generating the excitation signal. The present invention uses heterodyning techniques to produce two sinusoidal RF signals, a driving/reference signal 90 and a mixing signal 92. The present invention modulates the frequency of the two signals from 10 MHz to 200 MHz. One skilled in the art will appreciate that the present invention could vary the signals over a much larger frequency range. The preferred embodiment of the present invention generates the two signals with a frequency difference of 10 kHz. Another embodiment of the present invention generates the two signals using an adjustable offset frequency where the offset frequency is set through to the present invention's control software.

Paragraph at c. 11, l. 26-44.

Another, equivalent, way of writing the eigenfunction equations proceeds from the observation that:

$$\mathbf{G}\cdot\mathbf{A}^{-1}=\mathbf{cc} \tag{23}$$

and

$$\mathbf{S}=\mathbf{cs}\cdot\mathbf{A}=\boldsymbol{w}\cdot\mathbf{cc}\cdot\mathbf{T}\cdot\mathbf{A} \tag{24}$$

so that

$$\boldsymbol{w}^{-1}\cdot\mathbf{S}\cdot\mathbf{A}^{-1}=\mathbf{cc}\cdot\mathbf{T}=\mathbf{G}\cdot\mathbf{A}^{-1}\cdot\mathbf{T} \tag{25}$$

or

$$[\mathbf{G}^{-1}\cdot\boldsymbol{w}^{-1}\mathbf{S}\cdot\mathbf{A}^{-1}=\mathbf{A}^{-1}\cdot\mathbf{T}]$$

$$\underline{\mathbf{G}^{-1}\cdot\boldsymbol{w}^{-1}\cdot\mathbf{S}\cdot\mathbf{A}^{-1}=\mathbf{A}^{-1}\cdot\mathbf{T}} \tag{26}$$

Paragraph at c. 13, l. 10-40.

We may interpret the first of Eqns. (36) in terms of its column vectors as the representation of the r vectors of **cc** in the orthonormal basis of the r vectors of **U**:

$$\underline{c}_j = \sum_{i=1}^{r} \underline{U}_i P_{ij} \tag{37}$$

where $[c_j]$ $\underline{c}_j$ is the jth column of **cc**, $[U_i]$ $\underline{U}_i$ is the ith column of **U** and P_{ij} is the corresponding element of $\mathbf{P_U}$. From this expression and the linear independence of the $[c_j]$ $\underline{c}_j$ and $[U_j]$ $\underline{U}_j$, we see that $\mathbf{P_U}$ is invertible. Linear independence of the $[c_j]$ $\underline{c}_j$ requires that:

$$\sum_{j=1}^{r} \alpha_j \underline{c}_j = 0 \qquad \text{only if} \qquad \alpha_j = 0 \qquad \text{for all } j=1,\ldots,r. \tag{38}$$

From Eqn. (36), we see that Eqn. (37) implies:

$$\sum_{i,j=1}^{r} \underline{U}_i P_{ij} \alpha_j = 0 \ .$$

From the linear independence of the $[U_i]$ $\underline{U}_i$, we must have

$$\sum_{j=1}^{r} P_{ij} \alpha_j = 0, \qquad \text{for all } i = 1,\ldots,r. \tag{39}$$

Paragraph at c. 14, l. 1-12.

A second method for solving Eqns. (30) is to use Eqn. (31) to write the pseudo inverse of **G**:

$$\mathbf{Gpi} = \mathbf{V} \cdot \mathbf{C}_1^{-1} \cdot \mathbf{U}^T \tag{43}$$

so that we have:

$$\mathbf{G} \cdot \mathbf{Gpi} = \mathbf{U} \cdot \mathbf{U}^T \quad \text{and} \quad \mathbf{Gpi} \cdot \mathbf{G} = \mathbf{V} \cdot \mathbf{V}^T,$$

where $\mathbf{U} \cdot \mathbf{U}^T$ is [and] an NxN matrix and $\mathbf{V} \cdot \mathbf{V}^T$ is an MxM matrix, and each of these matrices is of rank r.

Equation 63, c. 15, l. 64.

$$[\eta = Tr(\mathbf{M}_{\mathrm{V}} \cdot \mathbf{U} \cdot \mathbf{U}_{\mathrm{T}})]$$

$$\underline{\eta = Tr(\mathbf{M}_{\mathrm{V}} \cdot \mathbf{U} \cdot \mathbf{U}^{\mathrm{T}})} \qquad (63)$$

Paragraph at c. 17, l. 1-6.

As the frequencies in ***w*** are large, we renormalize $\|\boldsymbol{w}^{-1} \cdot \mathbf{Sx}\|$ to equal $\|\mathbf{Gx}\|$ so as to avoid skewing the results in favor of the **Gx** data 138. This amounts to a [resealing] rescaling of the units for the frequencies and the lifetimes. This [resealing] rescaling of units is compensated once the lifetimes are found, so they are expressed in seconds.

Paragraph at c. 18, l. 63 to c. 19, l. 16.

FIG.10 illustrates the present invention's ability to identify and discriminate between individual overlapping spectral components in a target mixture. The present invention allows for the extraction of both the individual fluorescence spectra and lifetimes from a target mixture of fluorophores. FIG.10 illustrates the present inventions ability to differentiate spectra using a three-dye mixture of OXAZINE™ 720, 725 and 750. OXAZINE is a trademark of Excition, Inc. We mixed the dyes at equal concentrations of 3.3 μM. We recorded the emission spectra for an excitation signal wavelength of 640 nm and the laser modulation frequency was swept (modulated) from 10 MHz to 140 MHz at 5 MHz increments. The present invention extracted fluorescence lifetimes of 3.705 nsec for Oxazine 720 (196 on FIG.10), 1.979 nsec for Oxazine 750 [(] (198 on FIG.10), and 0.5588 nsec for Oxazine 725 (200 on FIG.10). The results from the present invention compared very well with the listed individual dye *fluorescence* lifetimes of 3.739 nsec for Oxazine 720, 2.014 nsec for Oxazine 750 and 0.9935 nsec for Oxazine 725. The individual spectra extracted for each dye from the mixture revealed spectral characteristics that matched with spectra obtained from the individual dyes.

In The Claims

Applicant is amending the claims in this patent under 37 CFR 1.173(b) and 37 CFR 1.173(d).

Amend claims 1, 6, 11, 16, and 21 as shown in this amendment.

Following is the currently pending claims with markings on separate pages with parenthetical statements that show the claim being amended, canceled, newly added, or left unchanged.

1. (Amended) An apparatus for fluorescence lifetime and spectral measurements, comprising:

a signal generator that generates the driving/reference signal, said driving/reference signal generator modulates the amplitude and/or the frequency of the driving/reference signal over time;

a signal generator that generates the mixing signal, said mixing signal generator modulates the amplitude and/or the frequency of the mixing signal over time;

an excitation signal generator that generates the excitation signal, the driving/reference signal drives said excitation signal generator;

a signal detector that detects the emitted signal;

a mixer that mixes the [emitted] mixing signal with the driving/reference signal and produces the processor reference signal;

a mixer that mixes the emitted signal with the mixing signal and produces the data signal; and

a processor that extracts the fluorescence lifetime and fluorescence spectrum of the emitted signal from the comparison of the processor reference signal with the data signal using a chemometric analysis.

2. The apparatus of claim 1 wherein the driving/reference signal and the mixing signal vary by an adjustable offset frequency.

3. The apparatus of claim 1 wherein said chemometric analysis extracts the fluorescence lifetime of the emitted signal from the phase difference between the processor reference signal and the data signal.

4. The apparatus of claim 1 wherein said chemometric analysis extracts the fluorescence spectrum of the emitted signal from the amplitude difference between the processor reference signal and the data signal.

5. The apparatus of claim 1 wherein said chemometric analysis further comprises a converging iterative solution.

6. (Amended) A system for fluorescence lifetime and spectral measurements, comprising:

means for generating the driving/reference signal, said driving/reference signal means modulates the amplitude and/or the frequency of the driving/reference signal over time;

means for generating the mixing signal, said mixing signal means modulates the amplitude and/or the frequency of the mixing signal over time;

means for generating the excitation signal, the driving/reference signal drives said excitation signal means;

means for detecting the emitted signal;

means for mixing the [emitted] <u>mixing</u> signal with the driving/reference signal to produce the processor reference signal;

means for mixing the emitted signal with the mixing signal to produce the data signal; and

a processor that extracts the fluorescence lifetime and fluorescence spectrum of the emitted signal from the comparison of the processor reference signal with the data signal using a chemometric analysis.

7. The system of claim 6 wherein the driving/reference signal and the mixing signal vary by an adjustable offset frequency.

8. The system of claim 6 wherein said chemometric analysis extracts the fluorescence lifetime of the emitted signal from the phase difference between the processor reference signal and the data signal.

9. The system of claim 6 wherein said chemometric analysis extracts the fluorescence spectrum of the emitted signal from the amplitude difference between the processor reference signal and the data signal.

10. The system of claim 6 wherein said chemometric analysis further comprises a converging iterative solution.

11. (Amended) A method for measuring the fluorescence lifetime and the fluorescence spectrum, comprising the following steps:

generating the driving/reference signal and modulating the amplitude and/or the frequency of the driving/reference signal over time;

generating the mixing signal and modulating the amplitude and/or the frequency of the mixing signal over time;

generating the excitation signal from the driving/reference signal;

detecting the emitted signal;

mixing the [emitted] mixing signal with the driving/reference signal and producing the processor reference signal;

mixing the emitted signal with the mixing signal producing the data signal; and

extracting the fluorescence lifetime and fluorescence spectrum of the emitted signal from the comparison of the processor reference signal with the data signal to measure using a chemometric analysis.

12. The method of claim 11 wherein the driving/reference signal and the mixing signal vary by an adjustable offset frequency.

13. The method of claim 11 wherein said chemometric analysis extracts the fluorescence lifetime of the emitted signal from the phase difference between the processor reference signal and the data signal.

14. The method of claim 11 wherein said chemometric analysis extracts the fluorescence spectrum of the emitted signal from the amplitude difference between the processor reference signal and the data signal.

15. The method of claim 11 wherein said chemometric analysis further comprises a converging iterative solution.

16. (Amended) A method of producing an apparatus for fluorescence lifetime and spectral measurements, comprising:

providing a signal generator that generates the driving/reference signal, said driving/reference signal generator modulates the amplitude and/or the frequency of the driving/reference signal over time;

providing a signal generator that generates the mixing signal, said mixing signal generator modulates the amplitude and/or the frequency of the mixing signal over time;

coupling an excitation signal generator that generates the excitation signal and the reference signal to said driving/reference generator;

providing a signal detector that detects the emitted signal;

coupling a first mixer to said excitation signal generator, said mixer mixes the [emitted] mixing signal with the driving/reference signal to produce the processor reference signal;

coupling a second mixer to said mixing signal generator, said mixer mixes the emitted signal with the mixing signal to produce the data signal; and

coupling a processor to said first mixer and said second mixer, said processor extracts the fluorescence lifetime and fluorescence spectrum of the emitted signal from the comparison of the processor reference signal with the data signal using a chemometric analysis.

17. The method of claim 16 wherein the driving/reference signal and the mixing signal vary by an adjustable offset frequency.

18. The method of claim 16 wherein said chemometric analysis extracts the fluorescence lifetime of the emitted signal from the phase difference between the processor reference signal and the data signal.

19. The method of claim 16 wherein said chemometric analysis extracts the fluorescence spectrum of the emitted signal from the amplitude difference between the processor reference signal and the data signal.

20. The method of claim 16 wherein said chemometric analysis further comprises a converging iterative solution.

21. (Amended) A program storage device readable by a computer, tangibly embodying a program of instructions executable by the computer to perform method steps for a method for measuring the fluorescence lifetime and the fluorescence spectrum, comprising the following method steps:

generating the driving/reference signal and modulating the amplitude and/or the frequency of the driving/reference signal over time;

generating the mixing signal and modulating the amplitude and/or the frequency of the mixing signal over time;

generating the excitation signal from the driving/reference signal;

detecting the emitted signal;

mixing the [emitted] <u>mixing</u> signal with the driving/reference signal and producing the processor reference signal;

mixing the emitted signal with the mixing signal producing the data signal; and

extracting the fluorescence lifetime and fluorescence spectrum of the emitted signal from the comparison of the processor reference signal with the data signal to measure using a chemometric analysis.

22. The program storage device of claim 21 wherein the driving/reference signal and the mixing signal vary by an adjustable offset frequency.

23. The program storage device of claim 21 wherein said chemometric analysis extracts the fluorescence lifetime of the emitted signal from the phase difference between the processor reference signal and the data signal.

24. The program storage device of claim 21 wherein said chemometric analysis extracts the fluorescence spectrum of the emitted signal from the amplitude difference between the processor reference signal and the data signal.

25. The program storage device of claim 21 wherein said chemometric analysis further comprises a converging iterative solution.

Status of the Claims under 37 CFR 1.173(c)

The following table summarizes the status of the claims of this application as of the date of this amendment:

Claim Number	Status
1	Pending, Amended
2	Pending
3	Pending
4	Pending
5	Pending
6	Pending, Amended
7	Pending
8	Pending
9	Pending
10	Pending
11	Pending, Amended
12	Pending
13	Pending
14	Pending
15	Pending
16	Pending, Amended
17	Pending
18	Pending
19	Pending
20	Pending
21	Pending, Amended
22	Pending
23	Pending
24	Pending
25	Pending

Remarks

This Preliminary Amendment amends the reissue application. In this amendment, the Applicant is amending the specification and the drawings. Additionally, the Applicant is amending the claims.

1. Specification

The Applicant is amending the specification in this patent under 37 CFR 1.173(b) and 37 CFR 1.173(d). These changes include ministerial changes, grammatical changes, and mistakes made by the US PTO during the printing of the patent. Applicant is not adding new matter with these amendments.

2. Drawings

Applicant is proposing to amend FIG. 8 under 37 CFR 1.173(b)(3). This amendment is to correct a misspelled word. The proposed amendment in red.

3. Claims

Applicant is amending the independent claims 1, 6, 11, 16, and 21 under 37 CFR 1.173(b) and 37 CFR 1.173(d). There is an incorrect element in the claims. This error occurred without deceptive intent of the Applicant. The support for this amendment to the claims as required under 37 CFR 1.173(c) is found in the patent at c. 6, l. 16-51 and in FIG. 6.

4. Summary

In view of the above, Applicant believes that each of the presently pending claims is in immediate condition for allowance or appeal. Accordingly, Applicant respectfully requests that the Examiner pass this application to issue.

Respectfully submitted,

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